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Emergence of Multiple Antibiotic Resistant Strains of Salmonella Species in Calabar

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Abstract: *A total of 213 Salmonella species isolated from patients at the Microbiology laboratory of UCTH between Jan 2008 -Dec 2012 were examined for resistance to 12 antimicrobial agents using the agar diffusion technique. In all, 83(38.97%) of the isolates were resistant to two or more antibiotics. The distribution of multiple antibiotic resistance (MAR) among these isolates show that 46(55.4%), 24(28.92%) and 13 (15.66%) were resistant to 2,3,4, antibiotics respectively. The distribution of MAR on yearly basis was 7 (28.0%), 11 (35.48%), 17 (39.53%), 21 (42.86%) and 29 (44.60%) in 2008, 2009, 2010, 2011, 2012 respectively. These result show a steady increase in MAR - Salmonella infections in Calabar. Antibiotic misuse by" health care professionals and quacks as well as the wide spread availability of fake and/or substandard drugs are the principal factors that are responsible for the emergence of MAR Salmonella species in this community.*

Keywords: *Emergence, Multiple Antibiotic Resistance, Salmonella Species.*

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I. Introduction

It was Huggulen in 1999 [1] who said that infectious diseases have decided more battles than the great generals. Infectious diseases have truly brought about great devastating consequences to mankind. Varieties of drugs have been used to combat the effect of these infectious diseases. Antibiotics are natural or synthetic drugs which inhibit or kill bacteria. This capacity makes them unique for the control of deadly infectious diseases caused by a large variety of pathogens. [2]. Antibiotic resistance is the ability of microorganisms to withstand exposure to antibiotics. This usually results in the inactivation of the antibiotics by the causative agent of the disease, this result in narrowing treatment options.

The causative agents of these infectious diseases have been seen to inactivate the effective working of these drugs. They inactivate these drugs in various ways viz: production of enzymes which destroy the active ingredient, developing an alternative pathway that by-pass the reaction inhibited by the drug or by developing an altered enzyme which can still perform its metabolic function but is much less affected by the drug than the enzyme in the susceptible organism [3, 4]. The problem of resistance is increased by the use of antibiotics as prophylactics, indiscriminate, inappropriate use for treatment of common viral infections to which they have no effect. The use of antibiotics in livestock feeds has led to wide spread contamination of meat and poultry by drug resistant bacteria such as *Salmonella* sp. [5, 6]. Multiple antibiotic resistance (MAR) is a phenomenon in which microorganisms resist the action of more than one antibiotic [4]. The transposon Tn21 and a group of closely related transposons (the transposon family) are involved in the global dissemination of antibiotic resistance in Gram negative bacteria. The molecular basis for their involvement is carried by the Tn21 family of mobile DNA element encoding a site specific system for the acquisition of multiple antibiotic resistance genes [7]. The frequency of occurrence of antibiotic resistance, especially, by *Salmonella* species has attracted renewed research interest and raised concerns among experts [8, 9, 10]. Salmonella is a member of the family enterobacteriaceae. They are gram negative and non-spore forming bacilli. Their natural habitat is the intestinal tract of humans and animals. Strains of Salmonella vary in their antimicrobial sensitivity pattern and their drugs resistance activity. Based on the observed trend, it is believed that Salmonella species may soon be resistant to all available antibiotics [11, 12, 13].

At present, the problem of antibiotic resistance is becoming more complicated and seems to defy the current solutions proffered, especially in sub-Saharan Africa where Salmonella seems endemic. It is even more worrisome with the knowledge that the rate of speciation is alarming [14]. In Cross River State, particularly Calabar, the State capital, the trend and rate of emergence of new strains have become life-threatening. This paper therefore seeks to highlight the new strains of Salmonella that have emerged within the period under review. With this information handy, more efficient strategies and agents can be developed to stem the tide of antibiotic resistance in this part of the world.

II. Materials and methods

2.1 Subjects

Patients used in this study were sampled from the Out Patient Department (OPD) of the University of Calabar with cases of Salmonella infections. All patients were classified with respect to age and sex.

2.2 Sample collection

2.2.2 Blood

Three millimeters of patients' blood samples were drawn out using a sterile syringe and drained into sterile tubes containing Brain Heart Infusion Broth. After mixing, the blood-broth mixture was incubated at 37°c for 48hrs" before the first subculture.

2.2.2 Stool

Stool samples from patients collected in sterile bottles were inoculated into selenite f-broth and incubated overnight.

2.3 Specimen culture

Blood samples from the Brain Heart Infusion broth were inoculated on to Cysteine Lactose Deficient Agar (CLED) using a sterile wire loop. These were incubated overnight at 37^0C. Stool sample was cultured by inoculating colonies from Selenite F-broth using a sterile wire-loop on to Desoxycholate Agar (DCA) and overnight incubation [15].

2.4 Antimicrobial sensitivity

The determination of antimicrobial sensitivity was accomplished using the National Association of Clinical Laboratory Standards of 2002. Isosenitest agar (Oxoid, UK) plates were freshly prepared and dried before use. Two to three discrete colonies of test organisms were inoculated into peptone water, incubated at 37°c for 6hrs and then used to flood the sensitivity agar plates. The excess was aseptically drained off. A pair of sterile forceps was used to transfer paper discs impregnated with varying concentrations of different antibiotics: Tetracycline (25mcg), Gentamicin, (10mcg), Ciprofloxacin (10mcg), Septrin (25mcg), Chloramphenicol (25mcg) and Streptomycin, (25mcg). The sensitivity and resistance were recorded based on their zones of inhibition. Control was done using standard strain of the organism with known sensitivity to the antibiotics tested [16].

III. Results and Discussion

The results show varying patterns of multiple antibiotic resistance by Salmonella species. There was a steady rise in the number of sub-types Table 1, shows the Salmonella species isolated within the study period. The two hundred and thirteen species isolated were of three types consisting of 135 species of S. *enterica* var typhimurium, 63 species of S. *enterica* var typhi and 15 species of S. *enterica* var paratyphi B.

The antibiotic resistance profiles of Salmonellae isolated between 2008 – 2012 is presented in Table 2.

The multiple antibiotic resistance patterns of Salmonella isolated between 2008 – 2012 is presented in Table 3.

The result of the percentage resistance of the various antibiotics used against the species of Salmonella is shown in Fig. 1. Ampicillin 60%, Streptomcin, 56.6%, Chloramphenicol 20.0%, while there was no resistance to ofloxacin, Ciprofloxacin and gentamicin. Variable resistance patterns were observed for the Salmonella species. Some species 90(113.3) were resistant to a single antibiotics, 46(51.8) were resistant to two different antibiotics, 24(27.9) were resistant to three antibiotics and 13(12.2) were resistant to four antibiotics. A total of one hundred and sixty six isolates were resistant to more than two antibiotics. It was also observed that multiple antibiotic resistance increased from 28.0% in 2008 to 44.6% in 2012.

The study revealed a very worrisome trend in multiple antibiotic resistance of Salmonella species during the period covered by the study. The pattern of resistance to the various antibiotics agrees with the works of [17, 18, 7, 13]. Acquired bacterial resistance to antibacterial agents is common in isolates from healthy persons and from persons with community acquired infections in developing countries [4, 12, 2].

Complex socio - economic and behavioural factors are associated with the problem of antibiotic resistance. Such factors include inappropriate use by patients and in clinical practice, poverty leading to purchase of small samples and use of the antimicrobials in sub-inhibitory concentrations encouraging the emergence of resistance. Several factors such urban migration and improper sewage disposal encourage exchange of antibiotic resistant organisms between people and the exchange of resistance genes among bacteria thereby increasing the prevalence of resistant strains.

IV. Conclusion

Multiple antibiotic resistance (MAR) of Salmonella species is a serious and life threatening problem. The organism is food-borne. Information on emerging antibiotic resistance is essential for clinical practice and rational policies against antibiotic resistance. Ensuring proper use of these agents, improvement of public sanitation and hygienic practice and fine-tuning hospital infection control strategies will stem the tide of this

phenomenon. Sadly the problem of antibiotic resistance will continue to escalate in developing countries, such as ours, if the above measures and more are not implemented and monitored to ensure compliance.

Table 1: Salmonella species isolated during study

Year	Resistant isolates (Number/%)			
	S. enterica var typhimurium	S. *enterica* var typhi	*S. enterica* var paratyphi B	Total
2008	18	5	2	25 (11.7)
2009	20	9	2	31(14.6)
2010	27	17	3	43(20.2)
2011	29	17	3	49 (23.0)
2012	41	21	3	65 (30.5)
Total	135	63	15	213 (100)

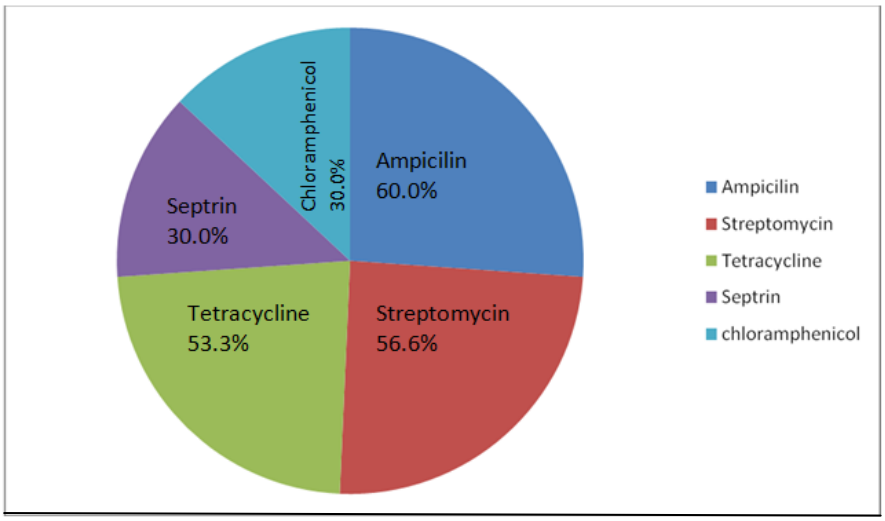
Table 2: Antibiotic Resistance Profiles of Salmonellae Isolated Between 2008 – 2012

Resistance profiles	Resistant isolates (Number/%)			
	Salmonella enterica var. typhimurium n = 135	Salmonella enterica var. typhi n = 63	Salmonella enterica var. paratyphi B n = 15	Total
R1	59(43.9)	27(42.9)	4(26.7)	90(113.3)
R2	37 (27.4)	7(11.1)	2(13.3)	46 (51.8)
R3	18 (13.3)	5 (7.9)	1 (6.7)	24 (27.9)
R4	10 (7.4)	3 (4.8)	0 (0.0)	13 (12.2)
Total MaR (>2AB)	65 (48.2)	15 (23.8)	3 (20.0)	83(92.01)
S all	11 (8.4)	21 (33.3)	8 (53.3)	40 (95)

Resistance to 1 antibiotic = R1; Resistance to 2 antibiotics = R2; Resistance to 3 antibiotics = R3; Resistance to 4 antibiotics = R4, sensitive to all antibiotics = S all.

Table 3: Multiple Antibiotic Resistant Salmonellae Species Isolated Between 2008 – 2012

YEAR	Resistant isolates (Number/%)			
	S. enterica Var typhimurium	S. *enterica* var typhi	*S. enterica* Var Paratyphi B	Total
2008	6(33.3)	1 (20.0)	0 (0.0)	7 (28.0)
2009	7(35.0)	3 (33.3)	0 (0.0)	10 (32.2)
2010	11(40.7)	5 (928.5)	1 (33.3)	17 (939.5)
2011	13 (41.3)	7 (43.7)	1 (33.3)	21 (42.9)
2012	18 (43.9)	10 (47.6)	1 (33.3)	29 (44.6)
	55 (40.7)	26 (41.3)	3 (20.0)	84 (44.1)



NIL - Ciprofloxin, Gentamicin, Ofloxacin

Fig 1: Percentage resistance of antibiotics used against Salmonella species

References

[1]. C. Huggelen (1999). Report of the first congress of the European society for emerging Infections. *Verhandlungen der deutschengecell Schaft for pathdogie.* 61(6): 649-659.

[2]. D. S. Djordjevic, Cain, A. K., Evershed, N. J, Falconer, L., Levings, R. S., Lightfoot, D. E. & Hall, R. M. (2009). Emergence and Evolution of multiple antibiotic resistant *Salmonella enteric* serovar paratyphln B. D-tartarate-utilizing strains containing SGI 1. *Antimicrobial Agents and Chemotherapy.* 53:231

[3]. K. A. Aldal, Harris, B. D. and Tomeki, K. J. (2000). New and Emerging Therapies in Infectious Diseases. *Dermatology Clinical* 18, 31-35.

[4]. K. Lee, Yong, D. & Chong, Y. (2004). Emergence of multi-drug resistant Salmonella enteric serovar typhi in Korea. *Antimicrobial Agents and Chemotherapy*. 48(11):4130-4135.

[5]. F. Bricarire, Caumes, E. J. and Do, P. H. (2000). Emerging and Re-emerging Infections Diseases a Challenge for Public Health *Schweizerische Rundschau Furmedlzln Praxis.* 89(4): 125-132.

[6]. M. Muhammad, Muhammed, U. L., Ambali a. mani, U. A., Azard, S. & Barco, L. (2010). Prevalence of Salmonella associated with chick mortality at hatching and agsusceptibility to antimicrobial agents. *Vererinary Microbiology* 140: 131-135.

[7]. V. C. Nwosu and Lodapo, J. A. (1999). Antibiotic Response and Plasmid Profile of Bacteria, *Current Topics in Microbiology* 39(5): 249 - 253.

[8]. I. S. Fisher (2004). Enter-net participants. Dramatic shift in the epidemiology of salmonella enterica serotype enteritidis phage type in Western Europe 1998-2003. *European Surveillance* 9:43-45.

[9]. E. Poirer, Watier, L,Espie, E., Weilli, F. X. De Valk H. and Desenclos J. C. (2008). Evaluation of Impact on human Salmonellosis of Control Meal Targeted to S *enteric* and S *typhimuruim* in Poultry Breeding Using time series Analysis and Intervention Models in France. *Epidemiology of Infections.* 136:147 - 1224.

[10]. S. Le-Hello, Brisabois, A., Accou-Demartin, M., Josse, A., Marault, M., Francart S. (2012). Food borne outbreak and non-motile Salmonella enteric Variant, France. *Emerging Infectious Diseases*. 18:132-134.

[11]. E. Jawetz, Melnick, 1, Adelberg, E. A. (1995). Medical Microbiology (20th Ed). Prentine Hall International Inc. new York PP 143-218.

[12]. P. Velge, Cloeckaert, A and Barrow, P. (2005). Emergence of Salmonella Epidemics: The Problems Related to *Salmonella enterica* Sertype enteritidis and Multiple Antibiotic Resistance in other Major Serotpes. *Veterinary Resistance*, INRA, EDP 26:267 - 288.

[13]. H. T. Van, Moulafis, G., Istivan, I. Iran, T., L. Peter, J. (2007). Development of Salmonella in Retail Raw Food Samples from Vietnam and their Characterization of their antibiotic Resistance. *Applied Environmental Microbiology* 73: 6885 - 6890.

[14]. Christopher M. Parry, Manar N Al-Mashhadani3, Samuel Kariuki4, Chisomo L. Msefula, Melita A. Gordon, Elizabeth de Pinna8, John Wain, Robert S. Heyderman Stephen Obaro, Pedro L. Alonso, Inacio Mandomando Calman A. MacLennan, Milagritos D. Tapia18, Myron M. Levine, Sharon M Tennant, Julian Parkhil and Gordon Dougan Intra-continental spread of human invasive *Salmonella* Typhimurium pathovariants in sub-Saharan Africa (2012) *Nat Genet*.44(11): 1215–1221.

[15]. M. Cheesebrough (2002). Antimicrobial Sensitivity Testing. In M. Cheesebrough (Ed), District Laboratory Practice in Tropical Countries (PEII). Cambridge: Cambridge University Press, 440.

[16]. National Committee for Clinical Laboratory Standards (NCCLS) (2002). M-100 Documents: *Performance Standards for Antimicrobial Susceptibility Testing*, 21, 105 - 119.

[17]. G. B. Soe, and Overturf, G. D. (1997). Treatment of Typhoid Fever and other Systemic Salmonellosis with Cefotaxime, Cefoperazone and other Newer Cphalosporins. *Review of Infectious Disease* 9:7- 19.

[18]. B. Ukwoma (1997). Salmonella Species now Resistant to Common Antibiotics Guardian Newspaper, July 24, p 26.

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